IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Chatterjee, D.K.

Appl. No. 09/558,421

Filed: April 26, 2000

For: **Mutant DNA Polymerases and Uses Thereof**

Art Unit: 1652

Examiner: Rao, M.

Atty. Docket: 0942.3600003/RWE/BJD

Declaration of Mary Longo

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Mary Longo, do hereby declare and say:

1. THAT, I, Mary Longo, hold the degree of M.S. A recent copy of my Curriculum Vitae, accurately listing my scientific credentials and work experience, is attached hereto as Exhibit A.

2. THAT, since 1979, I have been employed by Life Technologies, Inc. (LTI) (and now Invitrogen Corporation)[1], the assignee of the above-captioned application, in the capacity of Molecular Biologist. *See* Exhibit A.

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[1]Life Technologies, Inc. merged with Invitrogen Corporation on September 12, 2000, with Invitrogen Corporation being the surviving entity.

3. THAT, during my employment by LTI (and now Invitrogen Corporation), I worked under the supervision of Dr. Deb K. Chatterjee on a project involving the cloning, expression, and characterization of wild-type and mutant DNA polymerases.

4. THAT, I have reviewed my laboratory notebooks detailing my work on the project. Based on these laboratory notebook records and my recollection, the following activities involving my work, and relating to the DNA polymerase project, took place during the period from about October 16, 1994, until about September 8, 1995.

On or about July 12, 1995, I began a new project aimed at cloning nucleic acid encoding a *Tne* triple mutant lacking 3'-5' exonuclease activity, 5'-3' exonuclease activity, and the Phe to Tyr substitution in the O-helix into the plasmid ptrc99A. I restricted pUC*Tne*35FY (clone #1) with BspHI and restricted ptrc99A with NcoI and HindIII. The 4.1 kb band from the pUC*Tne*35FY mutant was cut out of the gel and frozen at -20° C overnight. This activity was recorded on page 157 of notebook 3959. A copy thereof is attached as Exhibit 1.

On or about July 13, 1995, I continued my efforts to clone the 35FY mutant of *Tne* polymerase. In this experiment I ethanol precipitated the restriction digests performed on July 12, 1995 and dissolved the products in TE buffer. The restriction products were further restricted with BspHI. The vector (ptrc99) and the 1.7 kb BspHI fragment were gel purified and DNA concentrations for both were determined. Clones #1 and #15 of pUC*Tne*35FY were pooled and dissolved in TE buffer. The pooled DNA sample was then restricted with BspHI and the

sample was frozen at -20° C overnight. This activity was recorded on page 157 of notebook 3959. A copy thereof is attached as Exhibit 2.

On or about July 14, 1995, I continued my attempts to clone the *Tne* 35FY mutant. In this experiment I ethanol precipitated the pUC35FY restriction product from July 13, 1995, and dissolved it in 1xR2 buffer. The mixture was then restricted with HindIII and the sample was applied to an LMP agarose gel. The 200 bp fragment was excised from the gel and frozen at -20° C. This activity was recorded on page 159 of notebook 3959. A copy thereof is attached as Exhibit 3.

On or about July 18, 1995, I continued my efforts to clone the *Tne* 35FY mutant. In this experiment I purified the DNA from the July 14, 1995 experiment via phenol-chloroform extraction method, and dissolved it in TE buffer. This activity was recorded on page 159 of notebook 3959. A copy thereof is attached as Exhibit 4.

On or about August 1, 1995, I began a new scheme for cloning the *Tne*35FY mutant of *Tne* polymerase into ptrc99A or a similar vector. The new scheme involved restricting pUC*Tne*35FY with HindIII, and filling in the ends with Klenow polymerase. This fragment was then restricted with SphI, and the 2 kb fragment was gel purified and then cloned into the SmaI/SphI site of pTTQ19. pTTQ19 was restricted with SmaI, and pUC*Tne*35FY was restricted with HindIII. Restrictions were run on an agarose gel to confirm the band pattern. The pTTQ19-SmaI digest was then subsequently digested with SphI. The pUC*Tne*35FY-HindIII fragment was treated with Klenow polymerase. The fragment was purified by phenol extraction, and

resuspended in 40 μl of R6 buffer. The sample was then restricted with SphI and run on an agarose gel. 2kb band was excised from the gel and frozen at -20° C. The fragment was then purified by the GENECLEAN method and dissolved in 10 μl TE buffer. Concentrations of both the pTTQ19-SmaI-SphI digest, and the 2kb HindIII-klenow-SphI fragment from pUC*Tne*35FY were determined by gel analysis. The two fragments were then ligated together at room temperature for 30 minutes. Jason Potter, another employee of LTI, transformed the ligation into DH10B competent cells, and plated the transformed cells on tet/amp plates. Cells were grown overnight. This activity was recorded on pages 181-183 of notebook 3959. A copy thereof is attached as Exhibit 5.

On or about August 2, 1995, I continued my cloning of the *Tne* 35FY mutant. On this day I picked eight colonies from plates inoculated on August 1, 1995 (*see* Exhibit 5), and inoculated them to liquid media. This activity was recorded on page 183 of notebook 3959. A copy thereof is attached as Exhibit 6.

On or about August 3, 1995, I continued my efforts to clone the *Tne* 35FY mutant. I isolated plasmid DNA from cells grown on August 2, 1995. Plasmids were restricted with SphI and EcoRI to confirm correct restriction patterns. This activity was recorded on page 183 of notebook 3959. A copy thereof is attached as Exhibit 7.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made

are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any registration resulting therefrom.

Further, declarant sayeth not.

Date: 11/26/01

Name: Mary Longo

Signature: Mary C. Longo

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SKGF1/25/98 dcw

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TECHNICAL SKILLS: Experienced in a wide variety of recombinant DNA techniques such as polymerase chain reaction, plasmid purification, cloning, restriction mapping, DNA sequencing, dot blots, Southern hybridization, batch purification, protein gels, in vitro transcription translation, and miscellaneous in vitro manipulations of DNA and RNA.

EXPERIENCE: Life Technologies, Incorporated.

Scientist
1994-present
Molecular Biologist in the Protein Engineering Analysis Division. Main responsibilities include feasibility and development of an in vitro transcription translation (IVT) system. Managed transfer of IVT to manufacturing. Other responsibilities were to clone and express genes of interest in *E.coli*, including protein expression. Lab supervisor for radiation safety program.

Associate Scientist III
1990-1994
Molecular Biologist in the Protein Engineering Analysis Division. Main responsibilities were to clone restriction enzymes and construct genomic libraries.

1987-1990
Biochemist in the Molecular Diagnostics Division. Main responsibilities were to design and conduct experiments exploring new methods of amplification and detection of infectious diseases such as human papilloma virus, hepatitis, and chlamydia.

Associate Scientist II
1981-1987
Biochemist in the Molecular Diagnostics Division. Responsibilities included laboratory development of biotechnology research products (e.g., Supercoiled Ladder and Uracil DNA Glycosylase). Played a major role in the development of Random Primer Amplification.

Associate Scientist I
1979-1981
Microbiologist in the DNA Products Group in the Operations Production Division. Laboratory responsibilities included isolation of plasmid DNA, centrifugation, column chromatography, and gel electrophoresis. Optimized techniques for yield and purity.

EDUCATION:

1985
MS degree in Nutritional Education from Hood College, Frederick, Maryland.

1979
BS degree in Food Science from University of Maryland, College

Park, Maryland.

AWARDS:

Received an employee achievement award in 2001 for developing the EXPRESSWAY™ In Vitro Protein Synthesis System.

Received an employee achievement award in 2000 for cloning Gateway clones.

Received company's highest award for technical innovation (David L. Coffin Award) in 1998, 1995, and 1992 for the development of BenchMark Protein Ladders, Method to Prevent Carryover Contamination of Amplification Reaction in Polymerase Chain Reaction (PCR), Improved Ladder for Determining the Size of DNA Molecules.

PATENTS:

Method for Production of Proteins. Patent pending.

Cloned *SstI/SacI* Restriction-Modification System. Issued 7/96.

Thermophilic DNA Polymerase from *Desulfurococcus tok*. Issued 5/96.

Cloned *NsiI* Restriction-Modification System. Issued 11/95.

Cloning and Expression of *AluI* Restriction Endonuclease. Issued 8/94.

Cloning and Expressing Restriction Endonucleases and Modification Methylases from *Caryophanon*. Issued 5/94.

PUBLICATIONS:

Smith, M., Longo, M., Gerard, G., and Chatterjee, D. "Cloning and Characterization of Genes for the PvuI Restriction and Modification System". (1992). *Nucleic Acids Research* 20(21):5743-5747.

Longo, M., Berninger, M., and Hartley, J. "Use of Uracil DNA Glycosylase to Control Contamination in Polymerase Chain Reactions". (1990). *Gene* 93:125-128.

Longo, M., and Hartley, J. "Storage of DNA". (1990). *Focus* 12(4):116-117.

Longo, M., and Hartley, J. "Comparison of Electrophoretic Migration of Linear and Supercoiled Molecules". (1986). *Focus* 8:3-4.

REFERENCES:

Available upon request.